Application No.: 09/898,398
Applicant: Hutchison
Filed: July 3, 2001
Page 3 of 15

Amendments to the Specification

Please replace the paragraph beginning at page 3, line 31 with the following amended paragraph:

~~FIG. 2 depicts graphs of observed PTH concentration (picomoles) versus spiked PTH (picomoles). Spiked PTH is defined as the addition of the specified amount of PTH in picomoles into the assay reaction.~~ FIG. 2A ~~illustrates~~ is a graph of observed PTH concentration (picomoles) versus spiked PTH (picomoles) using the Nichols Advantage Intact-PTH assay. FIG. 2B ~~illustrates~~ is a graph of observed PTH concentration (picomoles) versus spiked PTH (picomoles) using the bio-intact PTH assay ~~using~~ and the antibodies of the invention. Spiked PTH is defined as the addition of the specified amount of PTH in picomoles into the assay reaction.

Please replace the paragraph beginning at page 6, line 12 with the following amended paragraph:

~~FIG. 13 is a graph illustrating~~ FIGS. 13A and 13B illustrate the ratio of PTH to anti-PTH$_{1-13}$ antibodies as a function of the inhibitory PTH peptide. "1-13" indicates the use of a peptide consisting of amino acids 1-13 of PTH (SEQ ID NO: 1); "1-38" indicates the use of a peptide consisting of amino acids 1-38 of PTH (SEQ ID NO: 1); "1-34" indicates the use of a peptide consisting of amino acids 1-34 of PTH (SEQ ID NO: 1); "1-84" indicates the use of a peptide consisting of amino acids 1-84 of PTH (SEQ ID NO: 1); and "1-13" indicates the use of a peptide consisting of amino acids 1-13 of PTH (SEQ ID NO: 1).

Please replace the paragraph beginning at page 6, line 23 with the following amended paragraph:

~~FIG. 14 depicts~~ FIGS. 14A and 14B are graphs of PTH concentration (picomoles) as a function of retention time (minutes). FIG. 14A depicts the HPLC measurements for PTH standards (PTH$_{1-84}$; PTH$_{7-84}$; PTH$_{1-38}$; and PTH$_{1-34}$). FIG. 14B depicts the HPLC measurements for a "high" PTH group (e.g., greater than 200pg/ml). Data are shown for "intact PTH" (closed diamonds); PTH$_{1-84}$ (closed squares); and PTH$_{1-38}$ (triangles).

Application No.: 09/898,398
Applicant: Hutchison
Filed: July 3, 2001
Page 4 of 15

Please replace the paragraph beginning at page 6, line 31 with the following amended paragraph:

~~FIG. 15 depicts~~ FIGS. 15A and FIG.15B are graphs of PTH concentration (picomoles) as a function of retention time (minutes). FIG. 15A depicts the HPLC measurements for a "normal" PTH group. Data are shown for "intact PTH" (closed diamonds); PTH_{1-84} (closed squares); and PTH_{1-38} (triangles). FIG. 15B depicts the HPLC measurements for a "high" PTH group (e.g., greater than 500pg/ml). Data are shown for "intact PTH" (closed diamonds); PTH_{1-84} (closed squares); and PTH_{1-38} (triangles).

Please replace the paragraph beginning at page 7, line 8 with the following amended paragraph:

~~FIG. 16 depicts~~ FIGS. 16A and 16B are graphs of PTH concentration (picomoles) as a function of retention time (minutes). FIG. 16A depicts the HPLC measurements for samples without protease inhibitors. FIG. 16B depicts the HPLC measurements for samples with protease inhibitors. Data are shown for "intact PTH" (closed diamonds); PTH_{1-84} (closed squares); and PTH_{1-38} (triangles).

Please replace the paragraph beginning at page 7, line 15 with the following amended paragraph:

~~FIG. 17 depicts~~ FIGS. 17A-17C are graphs of PTH concentration (picomoles) as a function of retention time (minutes). FIG. 17A depicts the HPLC measurements for subject K. FIG. 17B depicts the HPLC measurements for subject G. FIG. 17C depicts the HPLC measurements for subject P. Subjects K, G, and P all had chronic renal failure

Please replace paragraph beginning at page 31, line 17 with the following amended paragraph:

To remove non-specific serum proteins, the columns were then washed with 0.1 M Na Acetate, 0.15 M NaCl pH 4.0, again monitoring the absorption at 280 nm. Specific antibodies to the peptide sequences were then eluted with 0.2 M Glycine pH 2.3. The low pH 2.3 elution by the

Application No.: 09/898,398
Applicant: Hutchison
Filed: July 3, 2001
Page 5 of 15

[[week]] weak buffer glycine dissociates the antibody from the covalently linked peptide to Sepharose 4-B. The final eluted antibodies to PTH1-13 are then neutralized with the addition of dilute 0.1 N NaOH to bring the pH back to 7.4.

Please replace the paragraph beginning at page 32, line 4 with the following amended paragraph

Antibody affinity purification columns consisting of $hPTH_{1-13}$, $hPTH_{13-34}$, and $hPTH_{39-84}$ are constructed with these peptides respectively liked to Sepharose 4B. The animal serum generated in the immunization sequence can be isolated for these respective antibodies in the following sequence. First antibodies to $hPTH_{39-84}$ are removed [[form]] from the sera, followed by removing antibodies to $hPTH_{1-34}$. Then the final affinity column of $hPTH_{1-13}$ is used to isolate the anti-$hPTH_{1-13}$ antibodies which recognize the bioactive conformationally correct N-Terminal sequence of hPTH. Although in our experiments the antibodies to $hPTH_{13-34}$ and $hPTH_{39-84}$ were first removed to prove there were no overlapping epitopes, it is possible to isolate the bioactive $hPTH_{1-13}$ conformational antibodies without removing the other antibodies first.

Please replace the paragraph beginning at page 32, line 26 with the following amended paragraph:

Acridinium as a "sulfonyl chloride ester" is crosslinked to the antibody of the invention by the reaction of the [[lysly]] lysyl moiety of the epsilon amino group of lysine in proteins, such as antibodies, to the acridinium ester. The reaction products are separated by size exclusion chromatography on Sepharose G-25 with 0.1 M Na Phosphate, pH 6.0.